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Synthesis and AMPA receptor antagonistic activity of a novel 7-imidazolyl-6-trifluoromethyl quinoxalinecarboxylic acid with a substituted phenyl group and improved its good physicochemical properties by introduced CF_3 group

Yasuo Takano,* Futoshi Shiga, Jun Asano, Wataru Hori, Tsuyosi Anraku and Takashi Uno

Discovery Research Laboratories, Kyorin Pharmaceutical Co., Ltd, Nogi-machi 2399-1, Simotsuga-gun, Tochigi 329-0114, Japan

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Abstract—We describe the synthesis, physicochemical, and biological properties of a novel series of 7-imidazolyl-6-trifluoromethyl quinoxalinecarboxylic acids with a substituted phenyl group attached through a urethane linkage at the C-7 position. We found that the introduction of trifluoromethyl group at the C-6 position brought about good biological activity and physicochemical properties. Among them, compound **9k (KRP-199)**, which has a 4-carboxyphenyl group, was found to possess high potency and selectivity for the AMPA-R in vitro and to exhibit good neuroprotective effects in vivo. Furthermore, the compound showed good physicochemical properties such as stability to light and good solubility in aqueous solutions.

Glutamic acid is a major excitatory neurotransmitter in the mammalian central nervous system, and its excitatory function is important for the life cycle of the neuron. In particular, overstimulation of post-synaptic glutamate receptors by release of excitatory amino acids (EAA) initiates neuronal death. Among the glutamate receptor antagonists, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R) antagonists appear to be free from side effects such as schizophrenia[1] and have shown effectiveness against neuronal death, even if administered post-ischemia.[2] In consequence, AMPA-R antagonists have been reported to be effective in the therapy of neurodegenerative disorders such as stroke, epilepsy,[3] head trauma, and Alzheimer's disease.[4]

Since the demonstration of the potent and selective AMPA-R antagonistic activity of NBQX,[4] several research groups have modified the quinoxalinedione structure. The numerous resulting compounds can be categorized as first-generation compounds of substituted simple quinoxalinedione structure, such as NBQX[4] and YM-90K,[5] and second-generation compounds with a hydrophilic substituent at the N-1 position of quinoxalinedione, such as YM-872[6] and ZK-200775[7] (Fig. 1). Some of these first- and second-generation compounds appear to be good antagonists, but have yet to be marketed as therapeutic agents.

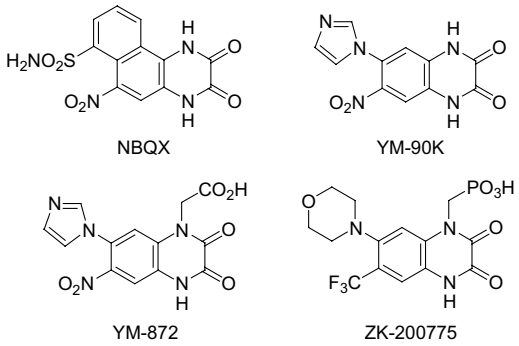

Figure 1.

* Corresponding author. Tel.: +81 280 56 2201; fax: +81 280 57 1293; e-mail: yasuo.takano@mb.kyorin-pharm.co.jp

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In a previous paper,[8] we reported the development of the novel 7-heterocycle-substituted quinoxalinecarboxylic acid **1** (GRA-293), which containing the carboxylic acid as a hydrophilic group and an imidazole in a molecule, as a third-generation AMPA-R antagonist. This compound bears a nitro group at the C-6 position and a 4-carboxylphenyl group attached through a urethane linkage to an imidazole at the C-7 position of 2-quinoxalinecarboxylic acid. GRA-293 shows excellent AMPA-R antagonist activity in vitro and in vivo compared with the known AMPA-R antagonists with quinoxalinedione structures. However, in continuation studies we found poor physicochemical properties, particularly instability to fluorescent light in neutral solution. The poor photostability of compound **1** precluded scaled-up synthesis and pre-formulation studies for parenteral use in the clinic. On the other hand, YM-90K and YM-872 with a quinoxalinedione nucleus also show instability under similar conditions[12] (see Table 3). The fact that these two quinoxalinedione compounds and our reported quinoxalinecarboxylic acid compound all show light instability in spite of their different structural nucleus led us to conclude that their common substituents, namely the nitro group at the C-6 position and the imidazole group at the C-7 position, might be the cause of the photo instability. Bigge et al. reported that an electron-withdrawing group at the C-6 position of quinoxalinediones is needed to interact with the AMPA-R because of the increased acidity of the proton of the amide moiety at the C-4 position.[9] Compound ZK-200775, which has a trifluoromethyl group instead of a nitro group as an electron-withdrawing group, has good AMPA-R antagonistic activity.[7] We attempted introduction of the trifluoromethyl group into the C-6 position on our quinoxalinecarboxylic acid nucleus (Fig. 2).

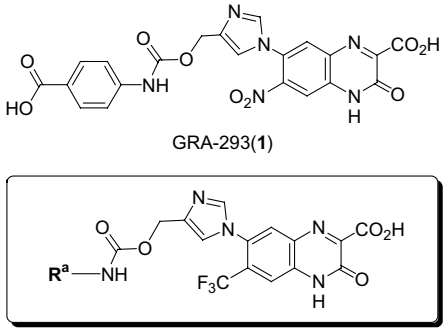
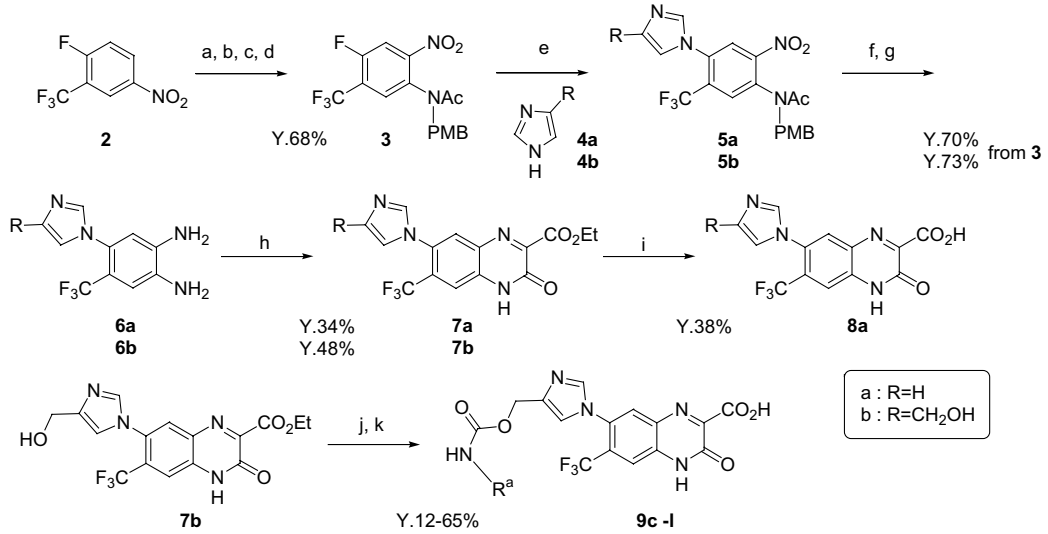


Figure 2.

Our research efforts have focused on designing and synthesizing novel quinoxalinecarboxylic acids possessing potent selectivity against the AMPA-R and good neuroprotective effects in vivo, as well as good physicochemical properties such as photostability in neutral solution. As a result, we discovered novel 6-trifluoromethyl quinoxalinecarboxylic acid compounds with a C-7 side chain. These compounds possess good AMPA-R antagonistic activity and are also photostable. In this paper, we wish to describe the synthesis of these novel 6-trifluoromethyl quinoxalinecarboxylic acid derivatives and their biological properties.

The syntheses of the novel 6-trifluoromethyl quinoxalinecarboxylic acid derivatives are outlined in Scheme 1. 4-Fluoro-3-trifluoromethylnitrobenzene **2** is the starting material for the key intermediates **7a**,**b**. After hydrogenation of **2**, protection of the amino group, selective nitrozation at the C-6 position and 4-methoxybenzyl (PMB) protection at the amide group were carried out in four steps in 68% yield. Compound **3** was treated with the commercially available imidazoles **4a**,**b**, followed by deprotection of the acetyl group and the PMB group and hydrogenation of the nitro group to give phenylenediamines **6a** and **6b** in 70% and 73% yield, respectively. Compounds **6a**,**b** were cyclized with diethyl ketomalo-



Scheme 1. H_2, 10% Pd–C, AcOH; (b) Ac_2O, Et_3N; (c) f.HNO_3; (d) PMB-Cl, K_2CO_3, DMF; (e) imidazole: **4a** or **4b**, Et_3N, DMF; (f) 4 N-HCl; (g) H_2, 10% Pd–C, EtOH–AcOEt; (h) diethyl ketomalonate, EtOH and then separate and recrystallization; (i) 6 N-HCl, AcOH; (j) R–NCO, Et_3N, DMF; (k) LiOH, EtOH–H_2O.

nate to afford ethyl quinoxalinecarboxylates **7a** and **7b** as key intermediates in 34% and 48% yield, respectively.

The nonsubstituted 7-imidazolyl quinoxalinecarboxylic acid **8a** was prepared by hydrolysis of ester **7a** with a yield of 38%. On the other hand, urethane-linked quinoxalinecarboxylic acids **9c**–**l** were prepared from the quinoxalinecarboxylate with hydroxymethyl group **7b** and a variety of isocyanates, followed by hydrolysis of the ethyl ester in 12–65% yield.

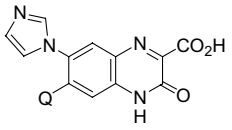
The affinities of the synthesized compounds for the AMPA-R and NMDA-R (*N*-methyl-D-aspartate receptor) were assessed by measuring their ability to displace, respectively, ^{3}H-AMPA (5 nM) and ^{3}H-CGS-19755 (10 nM) bound to crude synaptic membranes prepared from rat cerebral cortex.[10a,11] The AMPA-R antagonistic activity in vitro was confirmed by the inhibitory effect on AMPA-induced DC potential in rat cortical slices.[10b]

Initially, we investigated the effects of the C-6 substituent on AMPA-R affinity and photostability.[12] Table 1 shows a comparison of compounds with a trifluoromethyl group or a nitro group[8] at the 6-position on the quinoxaline nucleus, confirming that replacement of the nitro group by a trifluoromethyl group increased AMPA-R affinity 6-fold and imparted photostability. Based on these results, we conclude that for 2-quinoxalinecarboxylic acids with an imidazole group at the 7-position it is the 6-nitro group, that is, responsible for photo instability, and that 2-quinoxalinecarboxylic acids with a trifluoromethyl group at the 6-position will be very important core structures for design of novel AMPA-R antagonists.

We have already found that introduction of a benzene ring joined through a urethane linkage at the 4-position of the 7-imidazole group of 6-nitro-2-quinoxalinecarboxylic acid imparts high affinity and good selectivity for the AMPA-R.[8] Therefore, based on our knowledge of the 6-nitro-2-quinoxalinecarboxylic acid, we introduced the same side chain into 6-trifluoromethyl-2-quinoxalinecarboxylic acid in order to find an active compound with high AMPA-R affinity and better selectivity for the AMPA-R (Table 2).

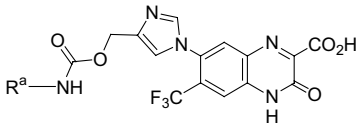
As a result, introduction of a benzene ring joined through a urethane linkage led to better AMPA-R affinity than that of the nonlinked compound (**8a** vs **9c**).

Table 1. Activity and stability of quinoxalinecarboxylic acid with 7-imidazole group



Compd	Q	AMPA-R affinity[10a] (K_i, nM)	Stability to light[12] (7000 Lx, 3 h)
8a	CF_3	86	(+)
10	NO_2	560	(−)

Table 2. Substituted 7-imidazole derivatives



Compd	R^a	AMPA-R affinity[10a] (K_i, nM)	Selectivity[11] $\left(\frac{\text{NMDA}-\text{R}}{\text{AMPA}-\text{R}}\right)$
1		22	>400
9c	Ph	23	160
9d	$PhCH_2$	190	NT
9e	3-Br–Ph	5.2	260
9f	4-Br–Ph	20	31
9g	4-Me–Ph	20	83
9h	4-MeO–Ph	30	57
9i	4-CF_3–Ph	29	>350
9j	3-CO_2H–Ph	35	>290
9k	4-CO_2H–Ph	16	>630
9l	3,5-Cl_2–Ph	13	240

NT, not tested.

However, the AMPA-R affinity of the benzyl compound, with a methylene group between the urethane linkage and the terminal benzene ring, was lower than that of the phenyl compound (**9c** vs **9d**), suggesting that extension of the methylene length at the terminal benzene ring would further reduce AMPA-R affinity. Introduction of a substituent into the terminal benzene ring of **9c** led to AMPA-R affinity similar to that of the nonsubstituted phenyl compound, with the exception that introduction of bromine into the 3-position of the benzene ring (**9e**) enhanced AMPA-R affinity. However, when the introduction of bromine, methyl, and methoxy group at the 4-position of the benzene ring the AMPA-R selectivity were reduced in spite of retaining good AMPA-R affinity level (**9c** vs **9f**–**9h**). On the other hand, introduction of electron-withdrawing groups such as trifluoromethyl and carboxyl groups at the 4-position of the benzene ring led to better selectivity, leading us to conclude that the preferred substituents were electron withdrawing. In particular, substitution by a carboxyl group at the 4-position, **9k**, led to both good affinity and high selectivity for the AMPA-R. It is noteworthy that conversion of the 6-nitro group of **1** to the 6-trifluoromethyl group of **9k** led to superior AMPA-R affinity and selectivity.

The neuroprotective effects of compounds **1**, **9k**, YM-90K, and YM-872 were examined with the permanent focal ischemia model in rats as described by Tamura et al.[13] The results are shown in Table 3.[14] The quinoxalinecarboxylic acid compounds showed better neuroprotective effects in vivo, as well as AMPA-R affinity in vitro, than those of the previously reported quinoxalinedione compounds. In comparison of biological activity between the 6-nitro compound **1** and the 6-trifluoromethyl compound **9k**, both compounds demonstrated highly potent activity in vivo. In particular, the neuroprotective effects of **9k** in the rat model were superior to those of the quinoxalinedione compounds, with a relatively low iv infusion rate of 2.5 mg/kg/h for 4 h, and its aqueous solubility was higher (compound **9k**,

Table 3. Pharmacological data of substituted 7-imidazolyl quinoxalinecarboxylic acid derivative

Compd	AMPA-R affinity[10a] (K_i, nM)	Selectivity[11] $\left(\frac{\text{NMDA}-\text{R}}{\text{AMPA}-\text{R}}\right)$	AMPA-R antagonism[10b] (DC potential)	Stability for light[12]	Protective effects in focal ischemia model[13,14] (dose; mg/kg/h for 4 h, iv)
YM-90K	100	430	(+)	(−)	2.8 (15)
YM-872	62	240	(+)	(−)	0.5 (30)
1	22	>400	(+)	(−)	3.0 (2.5)
9k	16	>630	(+)	(+)	2.7 (2.5)

8.29 mg/mL).[15] These characteristics of **9k** suggest its suitability as an injectable formulation for the treatment of acute cerebral ischemia. It is noteworthy that the photostability and receptor selectivity of **9k** are superior to those of **1**, even though both compounds possess a quinoxalinecarboxylic acid nucleus. It seems that introduction of a trifluoromethyl group at the C-6 position not only imparts potent AMPA-R affinity and high selectivity but also contributes to good physicochemical properties and to therapeutic efficacy in an animal model. Consequently, we believe that the trifluoromethyl-substituted quinoxalinecarboxylic acid **9k** is a useful structure for scaled-up chemical synthesis and pre-formulation studies for parenteral delivery.

In conclusion, molecular design of novel AMPA-R antagonists with a 6-trifluoromethylquinoxalinecarboxylic acid nucleus led to compounds with good biological activity and physicochemical properties. Investigation of structure–activity relationships and substance properties identified compound **9k (KRP-199)**[16] for further study. Finally, this research allowed us to develop superior AMPA-R antagonist candidates by replacing the 6-nitro group on the quinoxalinecarboxylic acid nucleus by a 6-trifluoromethyl group and exploited a new strategy to develop novel AMPA-R antagonists.

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12. *Stability test*: All test compounds were dissolved in 0.1 M sodium phosphate buffer (pH 7.4). Residual ratios were determined by HPLC analysis after irradiation for 3 h under a fluorescent light (7000 Lx) in a clear glass bottle. In the tables, (+) denotes more than 95% residual compound ratio, and (−) denotes less than 95%.
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14. *Evaluation of the rat focal ischemia model*: After 24 h of MCA occlusion as described by Tamura et al., brains were removed and sliced into five coronal (2-mm thick) sections with the use of a rat brain matrix (a manual slicer). Slices were placed in 2% (w/v) triphenyltetrazolium chloride (TTC) solution, and then in 10% (v/v) phosphate-buffered formalin. Tissue damage (areas not stained with TTC) was scored on a four-point scale (see figure A). Each test compound was administered by continuous iv infusion for 4 h, starting immediately after occlusion of the MCA. Control rats received saline only, and their four-point scale value was less than 1.0. As comparable compounds,

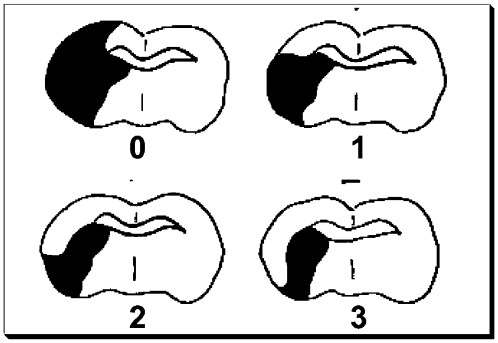

Figure A. Four-point scale

NBQX was purchased from Sigma–Aldrich chemical Co., and YM-90K and YM-872 were obtained following the above reported procedures (YM-90K, Ref. 5; YM-872, Ref. 6a).

15. *Solubility test*: The solubility of test compounds was determined in 0.1 M phosphate buffer (pH 7.4) at room temperature. After centrifugation, the concentrations in the supernatant were assayed by HPLC. The results at pH 7.4 were as follows: YM-90K, 1 mg/mL; YM-872, >5 mg/mL; compound **1**,4.86 mg/mL; Higuchi, T.; Shih, F. M.; Kimura, T.; Rytting, J. H. *J. Pharm. Sci.* **1979**, *68*, 1267.
16. The newly synthesized compound **KRP-199** has the following ^{1}H NMR and MS spectral characteristics: 7-(4-{[*N*-(4-carboxyphenyl)carbamoyloxy]methyl}imidazolyl)-3,4-dihydro-3-oxo-6-trifluoromethyl-quinoxaline-2-carboxylic acid (**9k**): white solid; mp 278–280 °C (dec); ^{1}H NMR (400 MHz, 25 °C, DMSO-d_6, ppm): δ 5.11 (2H, s), 7.55 (1H, s), 7.59 (2H, d, J = 8.8 Hz), 7.80 (1H, s), 7.86 (2H, d, J = 8.8 Hz), 7.89 (1H, s), 8.09 (1H, s), 10.2 (1H, s), 12.7 (1H, b s); HRMS (FAB$^-$) *m*/*z* calcd for $C_{22}H_{14}F_3N_5O_7$ (MH^-) 516.0767, found 516.0778. Anal. Calcd for $C_{22}H_{14}F_3N_5O_7{\cdot}2H_2O$: C, 49.02; H, 3.06; N, 12.99. Found: C, 49.01; H, 3.12; N, 12.68.